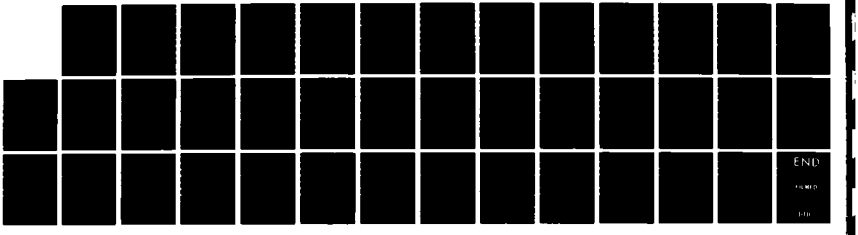
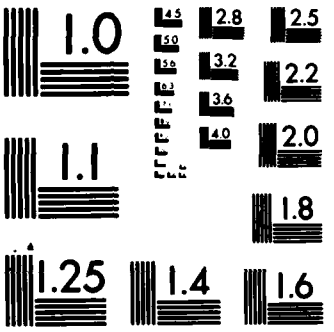
AD-A151 310 REACTION OF HUMAN SERA FROM JUEENILE PERIODONTITIS RAPIDLY PROGRESSIVE PE..(U) WALTER REED ARMY INST OF RESEARCH WASHINGTON DC J W VINCENT ET AL. 13 NOV 84 1/1

UNCLASSIFIED F/G 6/5 NL





(2)

When Data Entered)

AD-A151 310

ATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
Reaction of Human Sera from Juvenile Periodontitis, Rapidly Progressive Periodontitis, and Adult Periodontitis Patients with Selected Periodontopathogens

5. TYPE OF REPORT & PERIOD COVERED
Manuscript for Publication

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Jack W. Vincent, Jon B. Suzuki, William A. Falkler, Jr., and Willy C. Cornett

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U. S. Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, DC 20012

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
61102A 3M161102BS10 DA364

11. CONTROLLING OFFICE NAME AND ADDRESS
U. S. Army Medical Research & Development Command
SGRD-RMS
Fort Detrick, Maryland 21701

12. REPORT DATE
13 November 1984

13. NUMBER OF PAGES
Thirty-seven

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
This document has been approved for public release and sale; its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
None

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Periodontopathogens, Serology, Periodontal Diseases, ELISA

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
The levels of serum antibody reactive to selected periodontopathogens were determined in 183 clinically characterized patients: 35 healthy control, 50 juvenile periodontitis, 42 adult periodontitis, and 55 rapidly progressive periodontitis. Reactive antibody levels were determined utilizing an enzyme-linked immunosorbent assay with whole cell preparations of Bacteroides gingivalis, Capnocytophaga (Bacteroides) ochraceus, Fusobacterium nucleatum, and Actinobacillus actinomycetemcomitans (Y-4) serving as antigens. Increased

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

reactivity to B. gingivalis and F. nucleatum was observed in all three disease groups studied while antibody reactive to A. actinomycetemcomitans was increased in juvenile and rapidly progressive periodontitis. Antibody levels reactive to C. ochraceus in healthy subjects did not differ from those observed in any disease patient groups. Possible implications in the etiology and progression of the diseases coupled with environmental changes which occur in the econiche of the periodontal pocket are described.

Reaction of Human Sera from Juvenile Periodontitis, Rapidly Progressive Periodontitis, and Adult Periodontitis Patients with Selected Periodontopathogens†

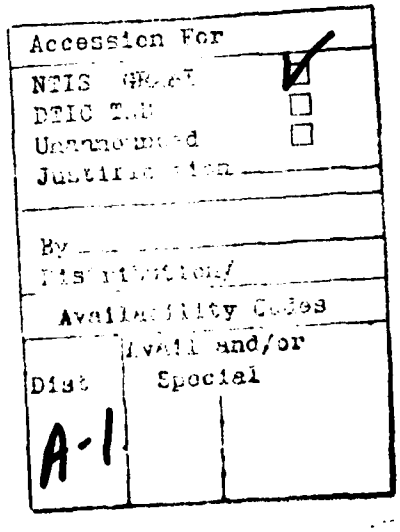
Jack W. Vincent,* Jon B. Suzuki,**
William A. Falkler, Jr.,** and Willy C. Cornett*

*US Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, DC 20307-5300

**Baltimore College of Dental Surgery
University of Maryland
Dental School
Baltimore, MD 21201

†Presented in part at the VI International Conference on Periodontal Research, Shefayim, Israel, June 19-23, 1984.

Reaction of Human Sera from Juvenile Periodontitis, Rapidly Progressive Periodontitis, and Adult Periodontitis Patients with Selected Periodontopathogens



ABSTRACT

The levels of serum antibody reactive to selected periodontopathogens were determined in 183 clinically characterized patients: 35 healthy control, 50 juvenile periodontitis, 42 adult periodontitis, and 55 rapidly progressive periodontitis. Reactive antibody levels were determined utilizing an enzyme-linked immunosorbent assay with whole cell preparations of Bacteroides gingivalis, Capnocytophaga (Bacteroides) ochraceus, Fusobacterium nucleatum, and Actinobacillus actinomycetemcomitans (Y-4) serving as antigens. Increased reactivity to B. gingivalis and F. nucleatum was observed in all three disease groups studied while antibody reactive to A. actinomycetemcomitans was increased in juvenile and rapidly progressive periodontitis. Antibody levels reactive to C. ochraceus in healthy subjects did not differ from those observed in any disease patient groups. Possible implications in the etiology and progression of the diseases coupled with environmental changes which occur in the econiche of the periodontal pocket are described.

The role of microbial plaque in the etiology of periodontal diseases has been established. Gingivitis has been produced in patients with healthy gingiva by the removal of all oral hygiene efforts and a state of gingival health restored after the reinstatement of rigorous oral hygiene.[1] These findings have been followed by numerous studies attempting to characterize microbial flora associated with gingival health and to elucidate shifts in the composition of this flora in various periodontal disease states. The major thrust of these studies has been toward the identification of a microorganism or groups of microorganisms which emerge as a significant proportion of the subgingival plaque in various disease states. The results of many of these studies have implied the importance of selected microorganisms in the etiology or progression of periodontal diseases. Based on an interpretation of the literature and on the capabilities and experience of the researchers, four microorganisms were selected for study.

Fusobacterium nucleatum is a Gram-negative anaerobic rod isolatable from the human gingival crevice region.[2] This microorganism increases in number in chronic gingivitis,[3] in early[4] and advanced periodontitis,[5,6] and in juvenile periodontitis (JP).[7] Numerous serological studies have demonstrated the significance of F. nucleatum in various disease states.[8-10] Higher levels of IgG and IgA antibody reactive to three typed

strains of F. nucleatum were observed in chronic periodontitis patients while the level of IgM antibody detected in chronic periodontitis patients showed no difference from healthy controls.[11] Cell fragments of F. nucleatum have been shown to attach to oral epithelial cells, gingival fibroblasts, and white blood cells,[12] suggesting a means of colonizing the periodontal pocket and mechanisms of subsequent tissue destruction by the activation of the alternate complement pathway[13] or the presence of endotoxin.[14]

Capnocytophaga (Bacteroides) ochraceus was described by Newman et al.[7] as a Gram-negative microorganism associated with the microbiota of periodontosis, an entity previously described by Orban and Weinman.[15] Newman et al.[7] describe "five periodontosis groups" of Gram-negative microorganisms in which C. ochraceus was designated as being in Group II. They further describe the ability of this microorganism to produce periodontosis-like lesions in monoinfected rats. Baer and Benjamin[16] have described the features of this disease entity as onset around puberty (11 to 13 years), increased incidence in females by a ratio of 3:1, and the presence of local factors not always correlated to the degree of periodontal destruction. Early in this disease process, the lesions progress rapidly but appear to slow with time[17] and may stop spontaneously or become superimposed with chronic periodontitis.[18,19] C. ochraceus was isolated from 9 of 10

plaque samples associated with gingivitis and 12 of 12 samples associated with JP, but in the latter disease it was isolated in approximately three times the number found in gingivitis.[20] Possible mechanisms of tissue destruction of C. ochraceus have been described as the in vitro bone-resorbing activity of lipopolysaccharides (LPS),[21] at a concentration of 10 μg/ml but not at 0.1 μg/ml, and the marked production of aminopeptidases which may be involved in bradykinin formation and the degradation of collagen fragments.[22] However, when compared to other suspected periodontopathogens, C. ochraceus proved to be inactive in promoting neutrophil chemotaxis and vascular exudation.[23]

Bacteroides gingivalis, as described by Coykendall et al.,[24] has been extensively studied to attempt to clarify its role in various periodontal disease states. Spiegel et al.[25] describe the absence of this microorganism from normal and gingivitis sites while being found at sites exhibiting bone loss. Antibody reactive with B. gingivalis has been found in 84% of normal adults and was presumed to be natural antibody serving a protective role.[26] These same researchers describe titers of IgG to be five times higher in adult periodontitis (AP) than in control subjects. Additionally, 5 of 10 generalized juvenile periodontitis patients had high levels of IgG antibody reactive with B. gingivalis. This disease, clinically similar to rapidly progressive periodontitis (RP),[27] has been characterized as

have been hampered primarily by the relatively small population groups for the various forms of disease studied. The purpose of the present study was to determine systemic antibody levels reactive to these selected periodontopathogens utilizing a standardized enzyme-linked immunosorbent assay (ELISA) with human sera obtained from periodontal disease-free individuals and from patients with various periodontal diseases.

MATERIALS AND METHODS

Patient Selection

Juvenile periodontitis patients were selected from the following sources: University of Maryland Dental School graduate and undergraduate clinics, The Johns Hopkins Hospital and Dental Clinics, and an author's (JBS) private practice limited to periodontics. Selection of patients was based on data obtained from clinical examinations, radiographic analysis, medical and dental histories, and interviews. Additional criteria for selection included the patient's desire to participate in the study, stable residence in the mid-Atlantic area, 20 or more teeth, and an age range of 12 to 26 years for JP and 12 to 35 years for RP. JP patients had a loss of attachment of at least 5 mm on any surface of at least four permanent incisors and first molars. RP patients had loss of attachment of at least 5 mm on the surface of at least 14 permanent teeth. Patients with systemic disease or who were pregnant were excluded from the study. Also, long-

exhibiting rapid destruction (seen most commonly in teenagers and young adults), extreme inflammation in the active phase of the disease, defects in either neutrophil or monocyte chemotaxis, and periods of exacerbation and quiescence. B. gingivalis purified LPS,[28] unidentified antigens,[29] and a specific extracellular antigen[30] have been shown to induce bone resorption in vitro, and a thio-dependent collagenolytic activity has been identified in the culture filtrate of B. gingivalis.[31]

Actinobacillus actinomycetemcomitans (Aa) has been isolated from the vast majority of subjects with juvenile periodontitis, but only infrequently from subjects with a healthy periodontium.[32,33] Numerous pathogenic mechanisms have been proposed to explain the role of this microorganism in periodontal disease states. One strain of Aa (Y4) contains a leukotoxin that inactivates human polymorphonuclear leukocytes and monocytes in the presence of normal human sera but is neutralized by sera from patients with JP.[34] In addition, both collagenolytic activity[35] and bone-resorbing activity[21] have been described.

In addition to the various microorganisms isolated in periodontal diseases, the role of the host defense mechanisms must be considered both as protective and as contributory to the disease processes. Humoral immunity has been evaluated by the study of locally produced or systemic antibody reactive to suspected periodontopathogens in various disease states.[36-40] Such studies

term medical therapy or the taking of medication six months prior to examination excluded patients from the study. Fifty JP and 55 RP patients were clinically evaluated, with this data summarized in Table 1.

Adult periodontitis patient selection was based on an age ranging from 36 to 54 years and 19 or more teeth. Tooth associated deposits were commensurate with the amount of periodontal destruction, but there was no specific pattern of bone loss.

Healthy subjects served as controls and were selected from periodontally healthy patients seeking restorative care. They included age- and sex-matched individuals from the population of dental students, dental hygiene students, faculty, and staff of the University of Maryland Dental School and The Johns Hopkins Hospital (Table 1). These individuals had periodic (every 3 to 6 months) prophylaxis, gingival[41] and plaque[42] indices of 0.5 or lower, and no historic, clinical or radiographic evidence[43] of periodontal disease.

Preparation of Serum

Venous blood was drawn into syringes, allowed to clot at room temperature, and subjected to centrifugation at 500 X g at 4°C. The serum was removed, complement inactivated at 56°C for 30 minutes, and frozen at -20°C.

Cultures

F. nucleatum (ATCC 10953), B. gingivalis (ATCC 33277), and B.

ochraceus (ATCC 27872) were obtained from the American Type Culture Collection, Rockville, MD. A. actinomycetemcomitans (Y4) was kindly supplied by Dr. Anne Tanner (Forsyth Dental Center, Boston, MA).

The microorganisms were cultured anaerobically utilizing the GasPak* anaerobic jar system. All cultures were harvested after 72 hours incubation by centrifugation at 10,000 X g for 20 minutes at 4°C and washed three times in 0.01 M phosphate buffer (PBS, pH 7.4) containing 0.15 M NaCl and stored at -20°C for future use. The F. nucleatum strain was cultured using a modified tryptone medium.[44] C. ochraceus and the Aa strain were grown in brain heart infusion (BHI) broth.* B. gingivalis was grown in BHI supplemented with hemin (5 µg/ml) and menadione (2 µg/ml). All microorganisms were tested for purity by subculturing on trypticase soy agar* with 5% defibrinated sheep blood, and typical biochemical reactions were verified with the API 20A system.†

Serological Evaluation by ELISA

All human sera were first diluted 1:20 in PBS for evaluation by a modification of an ELISA.[45] Briefly, whole cells of each of the microorganisms were suspended in a 60 mM carbonate buffer

---

*Baltimore Biological Laboratories, Cockeysville, MD

†Analytab Products, Plainsville, NY

(pH 9.6) and the concentration adjusted to an absorbance of 0.750 at 600 nm. A 200 µl aliquot was added to each well of 96-well microtiter plates[§] and incubated at 37°C for 3 hours. Peripheral rows were not utilized. The antigen preparations were then removed and each well was washed five times with 0.01 M PBS containing 0.05% Tween 20. Each well then received 200 µl of a 1% bovine serum albumin solution in a 60 mM carbonate buffer and incubated for 18 hours at 4°C. Following washing five times with PBS Tween 20, 100 µl of each serum sample (in duplicate) was placed in appropriate wells and the plates incubated for 30 minutes at 37°C. The plates were again washed five times with PBS Tween 20 and received 100 µl of a 1:600 dilution of peroxidase conjugated goat anti-human γ, α, and μ heavy-chain serum[¶] and the plates were incubated again at 37°C for 30 minutes. The plates were again washed five times, and 100 µl of an enzyme substrate was added to each well. This substrate was prepared by adding 1 ml of 1% (wt/v) O-phenylenediamine in absolute methanol to 99 ml of distilled water and 0.1 ml of 3% hydrogen peroxide. The plates were incubated in the dark for 30 minutes at room temperature, the reactions stopped by the addition of 50 µl of 8 N H_2SO_4, and the intensity of color resulting was determined

---

[§]Dynatech Laboratories, Inc., Alexandria, VA

[¶]Cappel Laboratories, Inc., Cochranville, PA

colorimetrically at 490 nm with an MR580 Microelisa Auto Reader.[§] Two wells in each plate served as controls receiving this same treatment, except that PBS was used in place of human serum. All assays were run at the same time on the same day in order to standardize incubation temperatures and buffers. The microtiter plates were all from the same lot, and sufficient anti-serum, substrate, and sulfuric acid were prepared from the same lots to be used for all assays.

RESULTS

The optical density (OD) of duplicate samples of 182 human-serum samples was determined when reacted with four Gram-negative microorganisms. The OD readings were automatically recorded after subtracting the OD obtained in the PBS control wells. In no case did the OD of the duplicate samples differ by greater than 0.01.

The mean OD for the 35 serum samples representing the control group varied from a high of 0.555 $\pm$ 0.252, when reacted with antigens of *B. gingivalis*, to a low of 0.125 $\pm$ 0.124, when reacted with antigens of *C. ochraceus* (Fig. 1). These data served as a baseline for comparison of antibody reactive to selected periodontopathogens observed in the sera of patients categorized in various disease groups. Statistical analysis was performed utilizing the *t* test for unequal *N*'s.

With the OD obtained by reacting these sera with the whole

cell preparations of B. gingivalis (Table 2), there was a statistically significant increase in reactive antibody found in patients with adult periodontitis ($P<.001$), juvenile periodontitis ($P<.001$), and rapidly progressive periodontitis ($P<.001$). The findings were similar for antibody reactive with the preparation of F. nucleatum (Table 3) (adult periodontitis, $P<.01$; juvenile periodontitis, $P<.05$; rapidly progressive periodontitis, $P<.001$). When reactive antibody to Aa was evaluated (Table 4), increased reactivity was seen in sera from patients with juvenile periodontitis ($P<.001$) and rapidly progressive periodontitis ($P<.001$), but not from those with adult periodontitis ($P>.05$). When serum antibody levels reactive with C. ochraceus were determined (Table 5), there was no significant difference when any disease group was compared with controls ($P>.05$).

When the patients with rapidly progressive periodontitis were further divided into two age groups, those 20 years of age and under (≤ 20) (N=9) and those over 20 years of age (>20) (N=46), the younger group demonstrated elevated antibody reactive to B. gingivalis, F. nucleatum, and Aa, while the older group demonstrated elevated antibody reactive to B. gingivalis and F. nucleatum, but not to Aa (Tables 6-8).

If an OD greater than two standard deviation units higher than the appropriate control group is deemed to indicate a positive reaction toward a specific microorganism, then 76% of JP and

25% of RP patients were positive for Aa, 66% of JP and 89% of RP patients were positive for B. gingivalis, and 20% of JP and 26% of RP patients were positive to F. nucleatum. There were no sera in any group which were positive to C. ochraceus. With RP patients, 11% of the >20 group and 100% of the ≤20 group were positive to Aa, while 78% and 93%, respectively, were positive to B. gingivalis. In the group of adult periodontitis patients, 79% were positive to B. gingivalis, 29% to F. nucleatum, and 0% to Aa.

DISCUSSION

The role of humoral immunity in any disease process may be twofold. In one, the complexing of antibody with antigen may activate complement by the classical pathway, finally resulting in lysis of Gram-negative microorganisms.[46] This process may also aid in phagocytosis or result in antibody-dependent cell-mediated cytotoxicity which may be protective or destructive in nature.[47] Also possible would be the second process in which this complexing of antibody and antigen may result in a hypersensitivity reaction via an immune complex disorder[48] which is initially characterized by a vasculitis as is seen early in periodontal disease. It seems reasonable to suggest that both the first, the protective role, and the second, the destructive role, are active in many disease processes. In addition, when evaluating circulating antibody levels reactive with a particular

pathogen in a disease process, specific kinetics are observed. Early in the disease process, antibody titers that can be detected are usually IgM and may not be significantly elevated. This may be due to insufficient time following sensitization to permit significant antibody production. Another possible explanation is that whatever antibody is produced during the acute phase of the disease rapidly binds available antigens and thus may not be readily detectable by serological means. However, if the test is repeated during convalescence, significant free antibody, usually IgG, is readily detectable. In this study, conjugated anti-human γ, α, and μ serum was utilized so as to establish total reactive antibody levels.

With this in mind, some interesting interpretations of this study can be discussed. The maturation of human dental plaque has been thoroughly described and consists of an early microflora, primarily Gram-positive and either aerobic or facultative anaerobic. If left undisturbed, there is a well-documented and orderly maturation to the point that in disease the flora is predominantly composed of Gram-negative anaerobic microorganisms. In addition, if one were to follow the ecological changes that occur as a healthy gingival sulcus develops into a periodontal pocket, one becomes aware that there is a continuing shift in which O_2 tension decreases, the reduction potential drops, and the substrates available to support microbial growth begin to

favor those organisms capable of asaccharolytic (protein) metabolism.

In patients with adult periodontitis as described in this study, it is obvious that opportunistic pathogens such as B. gingivalis and F. nucleatum are ideally suited for growth in the pocket econiche. Both organisms require the anaerobic environment and are proteolytic rather than saccharolytic, traits which are characteristic of the periodontal pocket. Mechanisms are known that may result in continuation of tissue destruction such as the activation of the alternate complement pathway by F. nucleatum and production of osteolytic substances by B. gingivalis. Metabolic by-products of both microorganisms are known to be toxic to host tissues. It is thus possible that, in a protective role, serum antibodies could bind these microorganisms and activate complement, and that the resulting cell lysis could expose these substances to surrounding host tissues.

The role of A. actinomycetemcomitans appears to be one of more complexity. The finding of low levels of antibody reactive with Aa in adult periodontitis patients, compared with the higher levels obtained from juvenile periodontitis patients, suggests a correlation of Aa with JP but not with AP. However, there is a strong suggestion of Aa's significance in JP patients as well as in some RP patients. This microorganism by its physiological characteristics is ideally suited for the environment encountered

at the onset of periodontal diseases. In vitro analysis indicates that Aa grows best in an atmosphere of air and CO_2 and is capable of metabolizing certain sugars and of peroxide breakdown.[6] Being thus suitable to survival and growth in the environment encountered at the onset of disease, the pathogenic potential of this microorganism is considerable. The leukotoxic activity,[34] osteolytic activity,[21] and typical membrane vesicles are characteristics that provide protection from the host and at the same time participate in tissue destruction. However, as the disease progresses, a more anaerobic environment, rich in protein, is created, which favors the stricter anaerobes such as B. gingivalis and F. nucleatum. This shift appears to be supported by the serological data from this study. The antibody response to Aa in RP suggests an even more complex reaction. In contrast to all the patients (100%) in the ≤ 20 group, who were able to mount a positive antibody response, only 11% of the >20 group were able to do so. This failure in the latter group may explain the tendency of RP to enter into remission in some patients, while tending to progress virtually unchecked in others. Such an apparent defect in the synthesis of protective antibody would permit the continuing contribution of Aa to the rapid destructive process by means of the pathogenic mechanisms previously described.

The possible influence of C. ochraceus in the etiology or

progression of any of the periodontal diseases in this study could not be supported. There were no statistically significant serological differences between any of the disease groups and control subjects. It must be remembered that this lack of antibody response by C. ochraceus may be due to its having an insignificant role or to its being a weak immunogen. This in no way precludes a role for C. ochraceus in an ecological sense, for it may provide growth factors supporting other microorganisms or contribute in some other way to the establishment of these complex polymicrobial diseases.

Aa may be of considerable importance in the initiation of JP and RP. Being somewhat aerotolerant, Aa may initially colonize the gingival crevice. Growth would result in a decreasing O_2 tension, thus aiding growth of anaerobes. The evident immunogenicity of Aa could result in host tissue destruction by previously described pathways and the leukotoxin would suppress the inflammability reaction, thus protecting Aa and other microorganisms. With the advent of attachment loss and osseous destruction, an environment is created more ideally suited for the growth of B. gingivalis and F. nucleatum, thus explaining the reported observation of JP with superimposed chronic periodontitis.

The lack of response in adult periodontitis to Aa, along with an increased response to B. gingivalis and F. nucleatum, is also supported by this study. The possible importance of C. ochraceus

in any of the disease processes could not be supported by this study. Lastly, the less frequent finding of a positive antibody response to Aa in older RP patients than in younger RP patients may lend some clue to the clinical observation of the disease going into remission in some patients, while progressing unabated to eventual tooth loss in others.

ACKNOWLEDGMENTS

The portions of this study conducted by the University of Maryland at Baltimore were supported in part by the Drew Award of the University of Maryland and the Omicron Kappa Upsilon-Charles Craig Fellowship Award of the American Fund for Dental Health. The authors appreciate the clinical assistance of Drs. Michael Siegel, Ray Lee, Tim Meiller, James Kassolis, Glenn Minah, Sue Noble, Thomas Daley, and Ruth Wooton, R.D.H.

MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the US Army Medical Department.

Send reprint requests to:

COL Jack W. Vincent, DC

Division of Research Sciences

U.S. Army Institute of Dental Research

Walter Reed Army Medical Center

Washington, DC 20307-5300

REFERENCES

1. Löe, H., Theilade, E., and Jensen, S.B.: Experimental gingivitis in man. J Periodontol 36:177, 1965.
2. Socransky, S.S.: Relationship of bacteria to the etiology of periodontal disease. J Dent Res, Suppl 2, 49:203, 1970.
3. Slots, J., Möenbo, D., Langebaek, J., and Frandsen, A.: Microbiota of gingivitis in man. Scand J Dent Res 86:174, 1978.
4. Darwish, S., Hyppa, T., and Socransky, S.S.: Studies of the predominant cultivable microbiota of early periodontitis. J Periodont Res 13:1, 1978.
5. Slots, J.: The predominant cultivable microflora of advanced periodontitis. Scand J Dent Res 85:114, 1977.
6. Tanner, A.C.R., Haffer, C., Bratthall, R.A., Visconti, R.A., and Socransky, S.S.: A study of the bacteria associated with advancing periodontitis in man. J Clin Periodontol 6:278, 1979.
7. Newman, M.G., Socransky, S.S., Savitt, E.D., Propos, D.A., and Crawford, A.: Studies of the microbiology of periodontitis. J Periodontol 47:373, 1976.
8. Evans, R.T., Spaeth, S., and Mergenhagen, S.T.: Bacteriocidal antibody in mammalian serum to obligatory anaerobic Gram-negative bacteria. J Immunol 97:112, 1966.

9. Kristoffersen, T.: Immunochemical studies of oral Fusobacterium. 6. Distribution of a group reactive antigen among some bacteria and occurrence of antibodies in human sera to this antigen. Acta Pathol Microbiol Scand 77:717, 1969.

10. Hofstad, T.: Antibodies reacting with lipopolysaccharide from Bacteroides melaninogenicus, Bacteroides fragilis, and Fusobacterium nucleatum in serum from normal human subjects. J Infect Dis 129:349, 1974.

11. Falkler, W.A., Jr., Lai, R., Vincent, J.W., Dober, L., Spiegel, C., and Hayduk, S.: The ELISA for measuring antibody reactive to Fusobacterium nucleatum in the sera of patients with chronic periodontitis. J Periodontol 53:762, 1982.

12. Falkler, W.A., Jr., Smoot, C.N., and Mongiello, J.R.: Attachment of cell fragments of Fusobacterium nucleatum to oral epithelial cells, gingival fibroblasts and white blood cells. Arch Oral Biol 27:553, 1982.

13. Hawley, C.E., and Falkler, W.A., Jr.: The anticomplementary activity of lipopolysaccharide preparations and sonicates from a strain of Fusobacterium nucleatum. J Periodontol Res 13:24, 1978.

14. Hampp, E.G., and Mergenhagen, S.E.: Experimental intracutaneous fusobacterial and fusospirochetal infections. J Infect Dis 112:84, 1963.

15. Orban, B. and Weinmann, J.P.: Diffuse atrophy of the alveolar bone (periodontosis). J Periodontol 13:31, 1942.

16. Baer, P., and Benjamin, S.: Resorptive lesions of the alveolar bone, in Periodontal Diseases in Children and Adolescents, pp. 139-181, Philadelphia, J.B. Lippincott Co., 1974.

17. Baer, P.N., and Socransky, S.S.: Periodontosis: case report with long-term follow-up. Periodont Case Rep 1:1, 1979.

18. Kaslick, R.S., and Chasens, A.I.: Periodontosis with periodontitis: a study involving young adult males. Oral Surg 25:305, 1968.

19. Kaslick, R.S., and Chasens, A.I.: Periodontosis with periodontitis: a study involving young adult males. II. Clinical, medical, and histopathologic studies. Oral Surg 25:327, 1968.

20. Eisenmann A., A.C., Eisenmann, R., Sousa, O., Slots, J.: Microbiological study of localized juvenile periodontitis in Panama. J Periodontol 54:712, 1983.

21. Iino, Y., and Hopps, R.M.: The bone-resorbing activities in tissue culture of lipopolysaccharides from the bacteria Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Capnocytophaga ochracea isolated from human mouths. Arch Oral Biol 29:59, 1984.

22. Nakamura, M., and Slots, J.: Aminopeptidase activity of Capnocytophaga. J Periodont Res 17:597, 1982.

23. Lindhe, J., and Socransky, S.S.: Chemotaxis and vascular permeability produced by human periodontopathic bacteria. J Periodont Res 14:138, 1979.

24. Coykendall, A.L., Kaczmarek, F.S., and Slots, J.: Genetic heterogeneity in Bateroides asaccharolyticus (Holderman and Moore 1970) Finegold and Barnes 1977 (Approved Lists, 1980) and proposal of Bacteroides gingivalis sp. nov. and Bacteroides macacae (Slots and Genco) comb. nov. Int J Syst Bacteriol 30:559, 1980.

25. Spiegel, C.A., Hayduk, S.E., Minah, G.E., and Krywolap, G.N.: Black-pigmented Bacteroides from clinically characterized periodontal sites. J Periodont Res 14:376, 1979.

26. Mouton, C., Hammond, P.G., Slots, J., Genco, R.J.: Serum antibodies to oral Bacteroides asaccharolyticus (Bacteroides gingivalis): Relationship to age and periodontal disease. Infect Immun 31:182, 1981.

27. Page, R.C., Altman, L.C., Ebersole, J.L., Vandesteen, G.E., Dahlberg, W.H., Williams, B.L., Osterberg, S.K.: Rapidly progressive periodontitis: a distinct clinical condition. J Periodontol 54:197, 1983.

28. Nair, B.C., Mayberry, W.R., Dziak, R., Chen, P.B., Levine, M.J., and Hausmann, E.: Biological effects of a purified lipopolysaccharide from Bacteroides gingivalis. J Periodont Res 18:40, 1983.

29. Patters, M.R., Landesberg, R.L., Johansson, L.-A., Trummel, C.L., and Robertson, P.B.: Bacteroides gingivalis antigens and bone resorbing activity in root surface fractions of periodontally involved teeth. J Periodont Res 17:122, 1982.

30. Vincent, J.W., Shaefer, D., Heath, J.R., III, and Falkler, W.A., Jr.: In vitro osteolytic activity of Bacteroides gingivalis extracellular antigen. Abstracts of the Annual Meeting of the American Society for Microbiology. 1984.

31. Toda, K., Otsuka, M., Ishikawa, Y., Sato, M., Yamamoto, Y., and Nakamura, R.: Thiol-dependent collagenolytic activity in culture media of Bacteroides gingivalis. J Periodont Res 19:372, 1984.

32. Slots, J., Zambon, J.J., Rosling, B.G., Reynolds, H.S., Christersson, L.A., and Genco, R.J.: Actinobacillus actinomycetemcomitans in human periodontal disease: Association, serology, leukotoxicity, and treatment. J Periodont Res 17:447, 1982.

33. Zambon, J.J., Christersson, L.A., and Slots, J.: Actinobacillus actinomycetemcomitans in human periodontal disease: Prevalence in patient groups and distribution of biotypes and serotypes within families. J Periodontol 54:707, 1983.

34. McArthur, W.P., Tsai, C.-C., Baehni, P.C., Genco, R.J., and Taichman, N.S.: Leukotoxic effects of Actinobacillus actinomycetemcomitans: Modulation by serum components. J Periodont

Res 16:159, 1981.

35. Robertson, P.B., Lantz, M., Marucha, P.T., Kornman, K.S., Trummel, C.L., and Holt S.C.: Collagenolytic activity associated with Bacteroides species and Actinobacillus actinomycetemcomitans. J Periodont Res 17:275, 1982.

36. Listgarten, M.A., Lai, C.-H., Evian, C.I.: Comparative antibody titers to Actinobacillus actinomycetemcomitans in juvenile periodontitis, chronic periodontitis and periodontally healthy subjects. J Clin Periodontol 8:155, 1981.

37. Ranney, R.R., Yanni, N.R., Burmeister, J.A., and Tew, J.G.: Relationship between attachment loss and precipitating serum antibody to Actinobacillus actinomycetemcomitans in adolescents and young adults having severe periodontal destruction. J Periodontol 53:1, 1982.

38. Kagan, J.M., Local immunity to Bacteroides gingivalis in periodontal disease. J Dent Res 59(DI):1750, 1980.

39. Kaslick, R.S., West, T.L., Singh, S.M., Chasens, A.I.: Serum immunoglobulins in periodontosis patients. J Periodontol 52:343, 1980.

40. Johnson, R.J., Matthews, J.L., Stone, M.J., Hurt, W.C., and Newman, J.T.: Immunopathology of periodontal disease. I. Immunologic profiles in periodontitis and juvenile periodontitis. J Periodontol 51:705, 1980.

41. Löe, H., and Silness, J.: Periodontal disease in pregnancy.

I. Prevalence and severity. Acta Odontol Scand 21:533, 1963.

42. Silness, J., and Löe, H.: Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 22:112, 1964.

43. Schei, O., Waerhaug, J., Lovdal, A., and Arno, A.: Alveolar bone loss as related to oral hygiene and age. J Periodontol 30:7, 1959.

44. Falkler, W.A., Jr., and Hawley, C.E.: Hemagglutinating activity of Fusobacterium nucleatum. Infect Immun 15:230, 1977.

45. Paxton, I.R.: Serological identification of Bacteroides species by an enzyme-linked immunosorbent assay. J Clin Pathol 32:294, 1979.

46. Nisengard, R.J.: The role of immunology in periodontol disease. J Periodontol 48:505, 1977.

47. Fundenberg, H.H., Stites, D.P., Caldwell, J.L., and Wells, J.V.: Basic and Clinical Immunology, ed 2, pp 272-273, Los Altos, Lange Medical Pub., 1978.

48. Page, R.C., and Schroeder, H.E.: Current status of the host response in chronic marginal periodontitis. J Periodontol 52:477, 1981.

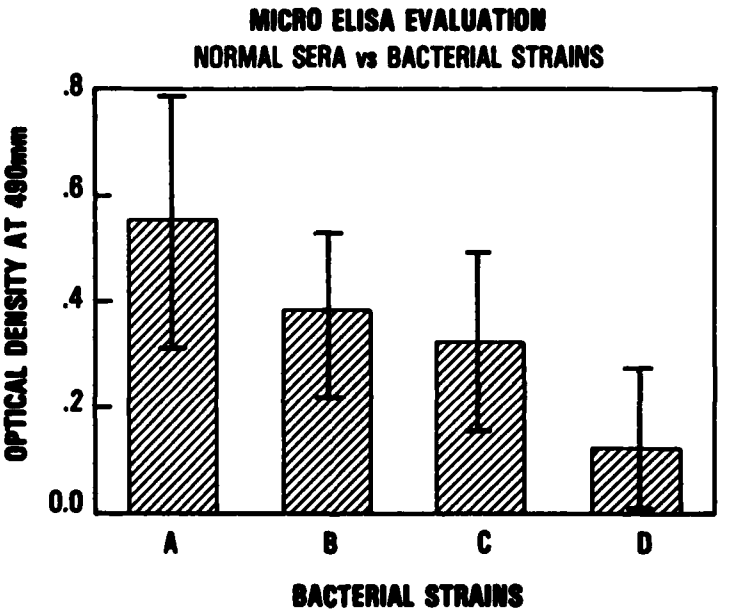


FIGURE 1. Mean OD of serum samples of control subjects when reacted with Bacteroides gingivalis (A), Fusobacterium nucleatum (B), Actinobacillus actinomycetemcomitans (C), and Bacteroides ochraceus (D).

TABLE 1. Clinical and Radiographic Features of Healthy Subjects (HS) and Patients with Juvenile Periodontitis (JP), Rapidly Progressive Periodontitis (RP), and Adult Periodontitis (AP)

Patient Groups	Age (yrs): Mean	Range	Teeth Present	Pocket Depth (mm)	Attachment Loss[a]	Bone Loss(%)[b]	Gingival Index[c]	Plaque Index[d]
HS	32.6 ±5.9	12-46	28.4 ±1.8	2.5 ±0.4	16±10	0	0.32 ±.3	0.36 ±.24
JP	16.8 ±3.91	12-26	29.6 ±1.67	5.7 ±1.4	210±55	44.6 ±8.9	1.6 ±.34	1.5 ±.45
RP	27.8 ±4.7	12-35	25.2 ±4.6	5.6 ±2.1	254±71	41.6 ±10.2	1.86 ±.45	1.45 ±.41
AP	44.2 ±8.2	36-54	24.3 ±5.2	5.8 ±2.4	261±90	46.1 ±12.1	1.8 ±.48	2.1 ±.52

[a]Loss of attachment = sum in mm from four surfaces per tooth (Glavind and Löe, 1967).

[b]Mean bone loss per affected tooth (>5mm loss of attachment) (Schei et al., 1959).

[c]Gingival index (Löe and Silness, 1963).

[d]Plaque index (Silness and Löe, 1964)

TABLE 2. Values Obtained for Serum Antibody Reactive to *Bacteroides gingivalis*

Patient Groups	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.555 ±.252	---	---
AP (N=42)	.953 ±.271	P<.001	79
JP (N=50)	.872 ±.307	P<.001	66
RP (N=55)	1.211 ±.279	P<.001	89

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 3. Values Obtained for Serum Antibody Reactive to *Fusobacterium nucleatum*

Patient Groups	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.384 ±.179	---	---
AP (N=42)	.514 ±.233	P<.01	29
JP (N=50)	.479 ±.257	P<.05	20
RP (N=55)	.560 ±.231	P<.001	26

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 4. Values Obtained for Serum Antibody Reactive to Actinobacillus actinomycetemcomitans (Y-4)

Patient Groups	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.329 ±.149	---	---
AP (N=42)	.371 ±.161	P>.05	0
JP (N=50)	.850 ±.294	P<.001	76
RP (N=55)	.455 ±.271	P<.01	25

*Standard deviation.
**Obtained using the t test for unequal N's.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 5. Values Obtained for Serum Antibody Reactive to *Bacteroides ochraceus*

Patient Groups	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.125 ±.124	---	---
AP (N=42)	.131 ±.122	P>.05	0
JP (N=50)	.162 ±.135	P>.05	0
RP (N=55)	.192 ±.124	P>.05	0

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 6. Values Obtained for Serum Antibody of Two Age Groups of RP Reactive with *Bacteroides gingivalis*

Patient Groups (RP by age)	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.555 ±.252	---	---
RP >20 (N=46)	1.199 ±.293	P<.001	78
RP ≤20 (N=9)	1.226 ±.202	P<.001	93

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 7. Values Obtained for Serum Antibody of Two Age Groups of RP Reactive with *Fusobacterium nucleatum*

Patient Groups (RP by age)	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.384 ±.179	---	---
RP >20 (N=46)	.548 ±.230	P<.001	22
RP ≤20 (N=9)	.621 ±.244	P<.001	29

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

TABLE 8. Values Obtained for Serum Antibody of Two Age Groups of RP Reactive with *Actinobacillus actinomycetemcomitans*

Patient Groups (RP by age)	Mean ± SD*	P value**	Percent positive†
HS (N=35)	.329 ±.149	---	---
RP >20 (N=46)	.380 ±.213	P>.05	11
RP ≤20 (N=9)	.839 ±.203	P<.001	100

*Standard deviation.
**Obtained using the *t* test for unequal *N*'s.
†Percent of sera demonstrating an OD greater than 2 SD units higher than HS sera.

END

FILMED

4-85

DTIC